THE ACTION OF CHLOROFORM UPON THE BLOOD VESSELS.

(AN EXPERIMENTAL RESEARCH.)

Thesis for the Degree of M.D.

by

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(Tracings of experiments in a separate book.)

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C O N T E N T S.

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INTRODUCTION

This research was undertaken to endeavour to determine the reason for the divergent results, obtained by previous observers, regarding the action of chloroform upon the blood vessels.

It will be noted, on examination of the work done on this subject, that different methods and different perfusion fluids were employed by different observers. Thus Schäfer and Scharlieb[1] perfused mammalian vessels with chloroform dissolved in Ringer's fluid. They observed that the effect of the drug is always in the direction of vaso-constriction in the vessels of the heart and limbs, whilst increased flow occurs through the kidney vessels (vaso-dilatation). On the other hand, Embley and Martin,[2] employing a pulsatory circulation apparatus and defibrinated blood, which was kept oxygenated by being passed through the lungs before being led to the organ under investigation, concluded that chloroform directly paralyses the neuro-muscular mechanism of both the kidney and bowel; and that a considerable part of the fall in blood pressure, caused by chloroform inhalation, may be accounted for in this manner.

In /

In a previous paper, on the effects of certain animal extracts upon the blood vessels (Argyll Campbell)[3] it was shown that pituitary extract, acting upon isolated blood vessels gives different results, if different physiological saline solutions be used.

In the present work, it is pointed out that the divergent results, previously given as the effect of chloroform upon the blood vessels, may be explained by the fact, that different perfusion fluids were used in the different series of experiments. Thus with defibrinated blood of arterial appearance results, opposite to those with Ringer's fluid, are obtained. Venous defibrinated blood gives the same results as Ringer's fluid. What difference exists between these fluids to cause the different results? It may be that they differ in their powers for carrying oxygen; certainly some condition is present in arterial blood which does not exist in Ringer's fluid and cyanotic blood.

PREVIOUS RESEARCHES.

1865.

Harley[4] states that there is a spasm of the small vessels, which can be readily seen to occur in the web of the frog's foot during chloroform anaesthesia; the vessels do not relax until the third stage is reached. Thus chloroform first stimulates and subsequently depresses the vaso-motor system.

1869-

Scheinesson[5] observed that, when an animal is under the influence of chloroform, no unusually strong irritation of the cervical sympathetic is necessary to cause a constriction of the vessels of the ear; that is the vaso-motor nerves, under chloroform, preserve their normal functions.

1874 -

Bowditch and Minot[6] found that, when an animal is under the influence of chloroform, irritation of the saphena nerve causes a much less marked rise of blood pressure than when the anaesthetic is not used. They also observed that inhalation of chloroform causes a decided depression of the blood tension. They excluded changes in the irritability of the afferent and efferent nerves to the vaso-motor centre and concluded that chloroform /

chloroform inhalation lowers the reflex irritability of the vaso-motor centre, thus diminishing the tendency towards rise of blood pressure from stimulation of a sensory nerve.

1879 -

Arloing[7] made observations on the rate of flow through the carotid artery, using the haemadromograph. He inferred that constriction of the arterioles is produced by chloroform.

1879 -

Newman[8] reporting on behalf of the committee on anaesthetics, of the British Medical Association, among other conclusions, mentioned that chloroform has a decided effect in reducing the blood pressure, and it does so much more rapidly and to a greater extent than ethidene. Occasionally he found that the pressure falls with great rapidity almost to zero. He attributed this to some capricious action of the drug on the heart.

In a second report he deals with the effect of anaesthetics on the pulmonary circulation of the frog. The anaesthetic was administered by means of a cannula placed in the glottis. The changes in the vessels were observed by placing a portion of the lung under a

microscope /

microscope. The blood pressure was not recorded. The conclusions in this report were that chloroform causes stoppage of the circulation both in the lungs and in the web of the foot; that the first change in the lungs is a diminution of flow of blood in the capillaries; that the flow becomes intermittent first in the capillaries, afterwards in the arterioles and subsequently in the larger vessels; that stoppage takes place in this order; sequence in recovery is the reverse; that circulation in the foot stops shortly after that of the lung; that the corpuscles are disintegrated or altered; that the epithelial cells of the lung meshes are no longer apparent.

1889 -

Hürthle[9] investigated the effect of chloroform upon the brain vessels, and observed that they are first dilated and then constricted. On inhalation of the vapour, the pressure in the aorta rises, whilst that in the"circle" does not experience a corresponding rise, but falls in spite of the increased pressure in the aorta. If chloroform be administered till death occurs, then the arteries of the brain are constricted again.

1890. -

Dastre[10] considered that the pallor of the face so /

so often noticed, points to vaso-constriction, as the effect of chloroform anaesthesia, rather than vaso-dilatation.

1890 -

Roy and Sherrington[11] state that, when administered by inhalation, chloroform causes marked contraction of the brain which is only in part due to the fall of general arterial pressure. This indicates vaso-constriction rather than dilatation. The brain volume was recorded by the plethysmographic method.

1890 -

Wood[12] found that chloroform abolishes the power of the circulatory mechanism to compensate for the influence of gravity in altered positions of the body.

1890 -

MacWilliam[13] states that the fall of blood pressure, caused by chloroform, is in its earlier stages due mainly to the depressing effect of the anaesthetic on the vaso-motor centre, often preceded by a slight stimulation; the later stages are associated with failure of the heart as well as of the vasomotor centre.

1891 -

The Hyderabad Commission[14] considered that the fall /

fall of blood pressure produced by chloroform is due to vasomotor paralysis and that it is beneficial to the heart.

1893. -

Hare and Thornton[15] concluded that the fall of blood pressure in chloroform anaesthesia is due to vasomotor and heart paralysis combined, the former being the chief factor.

1893. -

In his remarks on the action of chloroform Lauder Brunton[16] mentions that a gradual fall of blood pressure occurs in all cases, even in those where the narcotic is administered with perfect safety. He also describes an experiment in which chloroform was injected into the artery of a limb, producing rigor mortis and making the limb "as stiff as a board".

1893 -

Gaskell and Shore[17] did not think that chloroform produces local paralysis of the arterioles. They attributed the fall of blood pressure to heart failure alone, the vasomotor system not being paralysed but actually stimulated by the narcotic. They performed a series of cross-circulation experiments, to /

to prove the action on the vasomotor system. They did not investigate the action of chloroform upon the arterioles directly, but inferred from imperfect observations that whatever action chloroform has upon the blood vessels directly, it is insignificant compared with its much greater effects indirectly through the vasomotor centre.

1897 -

Leonard Hill[18] performed a series of experiments on the influence of gravity on the circulation. As regards chloroform he found that this anaesthetic, more than any other known agent, rapidly abolishes the vascular mechanisms which compensate for the hydrostatic effect of gravity. Chloroform abolishes these mechanisms by paralysing the splanchnic vasomotor tone and by weakening the action of"the "respiratory pump".

1900 -

Duplay and Hallion[19] investigating the time relations of respiratory and cardiac failure, concluded that death from chloroform is never due to failure of respiration, but to cardio-vascular paralysis.

1901 -

The committee on anaesthetics appointed by the British Medical Association[20] reported that "when danger /

danger occurs in the administration of chloroform, whatever the exact nature may be, there is abundant evidence that in a large proportion of cases the symptoms that are observed are those of primary circulatory failure".

1902 -

Embley[21] investigated the question of the action of chloroform upon the blood vessels, by employing simultaneous plethysmographic (intestine, kidney and spleen) and blood pressure records of the effects of the inhalation of known strengths of chloroform, firstly with the brain arteries open, secondly with the brain arteries closed. The effects of deep chloroform narcosis, upon vasomotor response to stimulation of the central end of the sciatic nerve and upon the vasomotor adjustment to rotation of the animal, from the horizontal to the vertical position, were also investigated. The effects of chloroform on the brain centres alone were also observed. By these methods, Embley concludes that chloroform causes a diminution of vascular tone of the arterioles; that this is most obvious when chloroform is practically kept out of the brain; that the central vasomotor system is stimulated at any rate for a time; that the cause of the fall of blood pressure is paralysis of the muscle - cells /

cells of the heart and of the arterioles.

1903 -

Sherrington and Sowton[22] have repeatedly noticed that the amount of perfused solution passing through the coronary circulation per unit of time, considerably diminishes soon after a chloroform - containing solution has replaced a chloroform - free solution. Since the pressure and temperature of the solutions supplied was the same in both cases, the diminution of flow indicates a vaso-constrictor action on the cardiac vessels; for the difference was so great as to be inexplicable by the loss of the power of the cardiac contractions or by a slight increase in the viscosity of the solution. They employed a modified Ringer's solution as their perfusing fluid.

1904 -

Schäfer and Scharlieb[1] perfused the vessels of a frog with chloroform dissolved in Ringer's solution. With the strongest solutions (1 in 200 to 1 in 500) a very marked constriction of the arterioles is the result. Although very slight when the dilution is considerable, they were able to substantiate constriction with solutions as weak as 1 in 20,000. They never had evidence of dilatation of the arterioles with any strength /

strength of chloroform.

They also perfused mammalian vessels with chloroform dissolved in Ringer's fluid. The organs, kidney, heart and limbs, were isolated and their vessels perfused. Chloroform solutions in all strengths (1 in 200 to 1 in 10,000) constricts the vessels of the heart and limbs. More dilute solutions are inactive. Chloroform solutions (1 in 200 to 1 in 1000) produce constriction of the vessels of the kidney. Weaker solutions (1 in 1500 to 1 in 20,000) dilate these vessels. More dilute solutions are inactive.

They showed that apocodeine which in sufficient dose abolishes the effect of adrenalin, does not abolish the effect of chloroform in producing vasoconstriction. From this they inferred that the action of chloroform upon the vessels, when perfused through the isolated organs, is a direct action upon the muscular tissue, and not, as in the case of suprarenal extract, upon the terminal apparatus of the vasomotor nerves.

1905 -

Embley and Martin(2) comfined their observations to determining the effect, upon the arteries of the isolated bowel and kidney, of chloroform in such quantities as may be present in the blood, when the air /

air respired contains 2 to 3% of chloroform vapour. They found that the dilatation produced is very marked, more especially upon the vessels of the bowel. They attribute the constriction obtained by Schäfer and Scharlieb[1] to the fact that these observers used strong chloroform solutions, the weakestof which would be in equilibrium with atmosphere containing 4.2% of chloroform. They state that the results of Schäfer and Scharlieb are not contradictory but supplementary to their own, and that in chloroform poisoning the dilatation would appear to be more or less confirmed to the splanchnic area.

In their experiments the perfusing fluid was defibrinated blood previously oxygenated by being passed through the lungs. The chloroform was administered in the air with which the lungs were rhythmically inflated by a mechanical pump.

1906 -

Sherrington and Sowton[23] perfusing the vessels of the limb of a cat with Ringer's fluid, oxygenated by bubbling oxygen through it, found that chloroform solution, 300 mg. to the litre (or about 1 in 300), exerts no obvious effect upon the rate of flow of the perfused fluid; change in the rate of flow through the limb vessels first becomes recognizable with solutions /

solutions of a concentration of 500 mg. to the litre (about 1 in 2000), constriction being produced. With stronger solutions (1 in 1000) there is first a transient diminution of flow, followed by an increase in rate of flow. This dilatation lasts as long as the chloroform solution is administered.

At the same time as they were conducting their perfusion experiments, they were tetanising the gastrocnemius muscle of the same limb.

They also showed that the presence of carbonic acid in the perfusing fluid, increases the action of strong solutions of chloroform (1 in 1000) in producing a greater flow through the vessels.

1908 -

Bayliss[24] states that the administration of chloroform is followed by a fall of arterial pressure which is usually attributed to failure of the heart's action, and no doubt this is the main factor; but he has several times observed in plethysmographic tracings of both intestine and limb in the initial stage before the pressure has fallen to any great extent, an unmistakable dilatation of the peripheral vessels. He states that this comes on too early in the fall of pressure to be due to the rise of venous pressure and /

and it gives place to a passive diminution of volume as the blood pressure continues its downward course; it is possible that this dilatation may be produced by direct action of the drug on the arterioles.

SUMMARY -

From the above account it is clear that the precise action of chloroform upon the blood vessels is by no means settled. According to Harley[4], Arloing,[7] Newman[8], Dastre,[10] Roy and Sherrington,[11] Sherrington and Sowton[22], and Schäfer and Scharlieb[1] chloroform produces vaso-constriction. According to Bowditch and Minot[6], Hürthle[9], MacWilliam[13], Wood,[12] the Hyderabad Commission,[14] Hare and Thornton[15], Leonard Hill[18], Embley[21], Enbley and Martin[2], and Bayliss[24] chloroform produces vaso-dilatation.

METHODS OF INVESTIGATING CHANGES IN THE BLOOD VESSELS.

The methods chiefly in vogue are the following, as detailed by Leonard Hill[25] :-

1. Direct observation of the colour of any part, or of the calibre of the arterioles as observed by microscope.
2. Thermometric observations of the temperature of any organ.
3. Estimation of the output of blood per minute from the efferent vein of an organ.
4. Record of velocity of blood flow in the artery supplying an organ, by using Chauvean's dromograph and similar instruments.
5. Estimation of the changes in volume of an organ by means of the plethysmograph.
6. Observations of the lateral pressure in the peripheral end of an artery as employed by Hürthle[9] in a research on the brain.
7. Simultaneous records of the lateral pressure in the afferent artery and the efferent vein of an organ.
8. Estimation of the capillary pressure.

Investigating the action of chloroform upon the blood vessels, Harley[4], Newman[8] and Dastre[10] employed /

employed the first of these methods; Arloing[7] the fourth; Roy and Sherrington,[11] Embley [21] and Bayliss[24] the fifth; Hürthle[9] the sixth. In all of these the blood vessels are kept in their normal positions in the body.

In addition to these methods, there are two means of dealing with the blood vessels completely isolated from the other structures of the body. The first of these, that of removing strips of the vessels and recording changes by actual measurement or by the movements of a lever on a drum, was employed by numerous observers, Meyer,[27] Langendorff,[28] Dale,[29] De Bonis and Susanna[30] and J. Pal[31] in their experiments with certain animal extracts (pituitary, suprarenal). The second of these methods, that of perfusing the blood vessels of an isolated organ and measuring the outflow from the vein, is more efficient because in this case the arterioles, the most important factor of the vascular system, are included as well as the larger vessels. Many different modes of perfusion have been employed. Langendorff's[34] apparatus is a common type. Other modes of perfusion were used by Brodie and Dixon,[32] Dale and Dixon[29] and Wiggers[33] in investigation of the action of extracts of the ductless glands upon the blood vessels. Sherrington and Sowton,[22] Schäfer and Scharlieb[11] and Embley /

Embley and Martin[2] were the only observers to employ the perfusion method in their experiments regarding the action of chloroform upon the blood vessels.

Bowditch and Minot[6], Harley[4], MacWilliam[13], The Hyderabad Commission[14], Hare and Thornton[15] and Leonard Hill[18] studied the effect of chloroform upon the whole vascular mechanism, i.e. the vasomotor centre, vasomotor nerves, heart and blood vessels. It is possible that the action of chloroform upon the entire mechanism may be different from its action on any one part of this mechanism, and the results of these observers cannot be assumed to give trustworthy evidence as to the action of the drug on the blood vessels alone. The only reliable method of settling this question is directly to study the action of the drug on the blood vessels, isolated as far as possible from other parts of the vascular mechanism. Now, the best method of doing this is to perfuse the vessels of the isolated organs. With the exception of Sherrington and Sowton[22], Schäfer and Scharlieb[1] and Embley and Martin[2], previous observers employed methods in which the blood vessels were not sufficiently isolated from other structures, consequently their results are of little value in deciding the present question.

Confining our attention to the experiments of those /

those who used the perfusion method, we find that the results of these experiments vary. It is my object to determine the reason for these differences. Now, in the experiments carried out by these observers, there were certain differences in technique. Thus, the organs perfused were different; the perfusion fluid, in which the chloroform was carried, varied; and the pressure conditions during perfusion also differed. The result of my investigations is that the pressure conditions have no influence, the organ selected for perfusion is, with one exception (kidney) a matter of indifference, while the fluid used for carrying the chloroform is of supreme importance.

In my research, I employed two methods, similar to those used by Schäfer and Scharlieb[1] and by Embley and Martin[2].

METHODS EMPLOYED IN THIS RESEARCH.

Two methods for perfusing the blood vessels of an organ.

METHOD No. 1. -

Procedure employed for perfusing with Ringer's fluid under constant oxygen pressure.— The apparatus rests in a large water bath, the temperature being kept at about 37°C. The apparatus consists of two glass reservoirs, one containing Ringer's fluid and the other a solution of chloroform in Ringer. The reservoirs are connected, each separately, with two of the limbs of a 3 - way stop-cock; to the other limb is attached a rubber tube leading to the cannula for the artery of the organ. This rubber tube leads up through a cork at the top of an inverted bell-jar, the top of the bell-jar being fixed to the bottom of the water bath; the bell-jar thus surrounded by warm water, acts as a warm air-chamber for the organ, the open end being covered over by a glass plate. The organ rests in a glass funnel fixed inside the bell-jar, the limb of the funnel passing through the cork top of the jar and also projecting through the bottom of the water bath.

The glass reservoirs are kept under constant oxygen pressure, produced as in Langendorff's heart apparatus by connecting the surface of the fluid in the reservoirs /

reservoirs with an oxygen bottle. The oxygen bubbles through the fluid and is prevented from exceeding a certain pressure (100 m.m. Hg. in most cases) by being connected with a mercury valve.

In order to detect any change in the pressure and thus to avoid fallacy, a manometer is connected with the fluid in the cannula. The tracing recorded by this is a straight line, since the pressure in the apparatus always remains the same.

By means of the 3 - way cock either pure Ringer or the solution of chloroform in Ringer could be passed through the apparatus at will. Since the pressure is the same in both reservoirs no alteration occurs in the pressure during this procedure, if the cock is turned rapidly. On thus changing the perfusing fluid from Ringer's fluid to chloroform solution a short interval elapses before the chloroform solution reaches the organ as there is still some Ringer's fluid (about 12 c.c.) between the chloroform solution and the organ. This interval is of advantage in that any errors, produced by manipulating the apparatus, are registered before the chloroform solution reaches the organ. The chloroform solution can perfuse the vessels as long as one desires, seeing there is a large supply in the reservoir.

The perfused fluid flowing from the vein of the organ /

organ is led away by the glass funnel, through the aperture in the bottom of the bath; it is collected in a tilter (Schäfer) which works by a see-saw action, each 6.2 c.c. of fluid being registered by transmission and recording tambours. The fluid, after leaving the tilter, is not used again, the Ringer and chloroform solutions simply flowing through the apparatus, not circulating.

METHOD - No. 2 -

Procedure employed for perfusing with defibrinated blood by means of a pulsatory circulation apparatus. (Fig. 1)

The apparatus used is a modification of Embley and Martin's [2] circulation scheme. Two test tubes (A and A^1), short but wide (Capacity 50 c.c. each) and connected below by a horizontal tube, act as a reservoir for the circulating defibrinated blood. The pump (B) for driving the fluid and for producing the pulsations differs from that used by the above observers. In my apparatus, the pump consists of two small oval pneumatic rubber balls (those used for working photographic shutters). They are connected with one another by a T - shaped glass tube. The pulsations are produced by eccentrics (C) as described by Embley and Martin.

The /

The other limb of the T - shaped tube is connected by a rubber tube with the upper end of the long limb of an h - shaped glass tube (D). The lower end of the same limb rests in one of the test tubes. The short limb of the h - shaped tube is connected with a buffer (E) (Embley and Martin) by means of a rubber tube. Both lower ends of the h - shaped tube are closed by rubber valves (Allenbury's baby-feeder valves slit down). In the lower end of the long limb the valve is directed upwards, thus allowing the defibrinated blood to pass up into the limb from the test tube but not back again. In the short limb the valve is pointed downwards, thus allowing the blood to pass on into the buffer but not back into the h - shaped tube. The short limb is in two pieces to facilitate the placing in and removing of the valve for cleansing purposes.

The blood never enters the rubber balls. They are filled with air. The downstroke of the eccentrics forces the blood into the buffer and the upstroke sucks up the blood from the reservoir, but never beyond the top of the long limb of the h - shaped tube.

The buffer (E) is connected by rubber tubes with a manometer (F) and with the cannula (G) for the artery /

artery of the organ. The pressure registered by the manometer is not constant but varies with the contraction of the blood vessels under experiment.

The organ is placed in a funnel, fixed on a stand inside an inverted bell-jar. Under the funnel is a tilter; and under each end of the tilter rests a glass funnel to catch the fluid as it flows from the tilter. From these funnels the blood is conducted back, by syphon action, to the test tube (A) and from this it passes to the test tube (A') containing the lower end of the h - shaped tube.

The blood in the test tubes is exposed to the air, but is also specially oxygenated by allowing a stream of oxygen to bubble slowly through the blood in A. Embley and Martin(2) were unable thus to oxygenate the blood owing to the formation of froth. I did not meet this difficulty unless the stream of oxygen was too rapid. In any case, if a froth does form, it never interferes with the experiment since it does not enter the apparatus, but remains at the surface of the blood in the test tube (A).

The apparatus is placed in a water bath, the temperature being kept at about 37°C. a thermometer is placed in the water, another is inserted into the arterial cannula. When properly working the temperature /

temperature in the bell-jar is almost the same as that of the water.

For an experiment a small quantity of the chloroform solution (saturated) in Ringer, previously mixed with some of the defibrinated blood of the same animal and kept in the water bath in a closed flask, is added to the blood in the test tube (A^1) connected with the pump. It is further diluted by this blood; it does not reach the organ for a short time after, as it has to pass through the h - shaped tube, the buffer and the rubber tube to the cannula, the capacity of its path being about 24 c.c. Any errors due to manipulating the apparatus during the addition of the chloroform solution are therefore recorded before the chloroform solution reaches the organ.

Every 8 c.c. which flows through the vein is registered by means of an electric recorder placed under one side of the tilter. The tilter used in this apparatus is slightly larger than that used in the first apparatus.

The chloroform solution was exposed to the air in the test tube (A^1) for a short time before it entered the h - shaped tube; therefore in reality the strength of the chloroform solutions were slightly weaker than given in the figures. Relatively the strengths /

strengths are correct. As the point under consideration was the comparative effects with different perfusion fluids, this disadvantage was not considered important. In the first apparatus, this defect is not present.

After the mixture had circulated once and had reached the organ a second time it was found inert, probably because it had been exposed, in small quantities at a time, to the air in the funnel, in the tilter, in the glass funnels under the tilter and in the reservoir. In this course the chloroform would evaporate almost completely as it takes a considerable time to pass through these parts of the apparatus and the whole is at a raised temperature.

Only about 120 c.c. of defibrinated blood are required to fill this circulation apparatus. A large cat yields about this quantity of blood.

After an experiment has been performed with defibrinated blood as the perfusing fluid, the blood can be slowly run out from the apparatus by means of a rubber tube leading from the glass funnels; at the same time Ringer's fluid is added to the reservoir to replace the blood until pure Ringer fills the whole apparatus and the organ as well. Then an experiment can be performed, with Ringer as the circulating fluid, on the same organ, and the two results /

results compared.

The animals used were chiefly cats. A few dogs were also employed; in one case a rabbit.

To kill the animal it is first etherised, as small a quantity of ether as possible being used. It was considered better not to employ chloroform for this purpose. The carotid artery is opened and the animal bled to death. As the blood flows out it is caught in a glass dish and defibrinated at once, by beating it vigorously with a bunch of feathers. It is then filtered through muslin and placed in the warm bath. It was used either undiluted and called 100% defibrinated blood, or was mixed with varying proportions of Ringer's fluid; thus thirty parts defibrinated blood and seventy parts Ringer made a 30% defibrinated blood solution.

The organ for perfusion is rapidly removed from the same or another animal and immediately placed in the apparatus.

The Ringer's fluid had the composition :-

NaCl .9 per cent.

KCl .1 " "

Calcium phosphate to saturation.

A standard solution of chloroform (Sp.G 1.49) in /

in Ringer's fluid was kept. The Ringer was saturated by being shaken up with and kept over an excess of chloroform at room temperature, and was considered to contain one part of chloroform in 200 parts of Ringer, this being the amount water will take up at ordinary room temperature (15°C.)

When required this was diluted by adding Ringer's solution or defibrinated blood of the same strength, as was being employed to perfuse the organ under investigation.

RESULTS OBTAINED.

A. With the vessels of the limbs (rabbit, cat.)

Lister[26] in his original experiment, showed that not only a drop of chloroform when applied but also the vapour of chloroform is able to produce hyperaemia in the web of the frog's foot. Lauder Brunton[16] injected pure chloroform into the artery of a limb, thus producing rigor mortis and making the limb as stiff as a board.

Schäfer and Scharlieb[1] found that chloroform solutions produce constriction in the vessels of the limbs of the rabbit and cat.

Sherrington and Sowton[23] obtained vaso-constriction in the limb of a cat when perfusing it with chloroform solution (1 in 3000). With stronger solutions (1 in 1000) vaso-dilatation is produced after a transient vaso-constriction.

The cannula was tied into the subclavian or femoral artery of a cat or rabbit. If Ringer's fluid be the perfusing fluid, chloroform solutions (1 in 600 to 1 in 10,000) produce constriction of the vessels. Whether oxygen be specially bubbled through the Ringer does not apparently make any difference to the result; it seems that Ringer does not take up oxygen in a sufficient amount, for venous blood gives the same /

same result as Ringer. With Ringer's fluid, a solution of chloroform (1 in 3000) reduced the rate of flow from the veins of the hind limb of a rabbit from 32 c.c. per min. to 20 c.c. per min. After the action had passed off under the influence of fresh Ringer's fluid the flow returned to 32 c.c. per min.

If defibrinated blood be the perfusing fluid the presence or absence of oxygen makes a great difference. If oxygen be present in excess a marked dilatation is observed with all strengths (1 in 1000 to 1 in 3000) of chloroform solution; thus the rate of flow from the veins of the fore-limb of a cat perfused with undiluted defibrinated blood (100%) was increased from 22 c.c. per min. to 26 c.c. per min. by the action of chloroform (1 in 3000). After the action had passed off the rate was 22 c.c. per min. The rates of flow before, during and after the addition of chloroform (1 in 3000) in an experiment with 30% defibrinated blood, were 32 c.c., 40 c.c. and 32 c.c. per min. respectively.

On the other hand should oxygen be absent a marked constriction is observed with the same strength of chloroform(1 in 3000); thus, the rate of flow was reduced from 24 c.c. per min. to 20 c.c. per min., the perfusing fluid being defibrinated blood (50%) venous /

venous in appearance.

Very strong solutions of chloroform (1 in 600 to 1 in 1000) produce constriction also with specially oxygenated defibrinated blood, but the blood is altered markedly by such strong solutions and becomes venous in appearance. This fact probably accounts for the constriction. In some cases where oxygen was not specially supplied, the blood seemed to take up enough oxygen from the air to appear arterial in colour. In any case as soon as the blood becomes venous the constricting effect of chloroform is very marked.

When oxygen is specially added the blood keeps its arterial appearance. But after the experiments have progressed for a time, defibrinated blood seems to lose its power of absorbing oxygen, even if specially bubbled through it, and becomes permanently venous. The addition of the chloroform for the experiments is probably the cause of this. There is however, usually plenty of time to do many experiments with the same specimen of defibrinated blood and with the same organ before the blood attains this permanent venous condition.

The rate of attaining this permanent cyanotic condition depends upon the organ and the strength of the /

the chloroform solution added to the blood, When the limbs are being used this condition is produced less rapidly than when the liver is under investigation.

Undiluted defibrinated blood gives the same results as a 20 per cent. solution of defibrinated blood in Ringer, although the greater the power for carrying oxygen the more marked is the dilatation produced.

The reason why constriction is obtained with strong solutions of chloroform is not that rigor mortis is produced. A solution of chloroform (1 in 700) reduced the flow from the veins of a fore limb of a cat from 28 c.c. to 20 c.c. per min., the vessels being perfused with Ringer. After the action had passed off the flow was 32 c.c. per min. Then the experiment was repeated (1 in 700 chloroform) and the rate was again reduced, this time from 32 c.c. to 16 c.c. per min. The chloroform was in the vessels for two minutes, yet the action passed off under fresh Ringer's solution and the rate became 32 c.c. per min. again. Thus rigor mortis is not produced under the above conditions.

Certainly rigor mortis occurs if the chloroform be perfusing for too long a time, at the above concentration. I found it difficult to produce rigor mortis /

mortis with solutions weaker than 1 in 2000. In the kidney it will be seen that chloroform solutions as strong as 1 in 1000 produce marked dilatation, not rigor mortis, even if the chloroform be allowed to act for ten minutes.

(Figs. 2 - 11) .

(B) With the vessels of the lungs (rabbit, cat).

Newman[8] concludes that chloroform produces slowing and ultimate stoppage of the circulation in the lungs, first in the capillaries, second in the arterioles and subsequently in the large vessels.

The pressure under which the perfusion of the lungs was carried out in my experiments varied from 20 m.m. to 40 m.m. Hg.

The same results were obtained as with the limbs. With oxygenated defibrinated blood (50%) the action of a solution of chloroform (1 in 5000) was to increase the rate of flow from the pulmonary veins from 26 c.c. to 40 c.c. per min. After the action had passed off the flow was 32 c.c. per min. With a weaker solution (1 in 10,000) and under similar conditions the flow was increased from 20 c.c. to 26 c.c. per min. After this experiment the flow returned to the rate of 20c.c. per min.

Marked /

Marked constriction is obtained with Ringer's fluid and with venous defibrinated blood, as the result of the addition of chloroform (1 in 300 to 1 in 10,000.) Thus a solution of chloroform (1 in 3500), added to the perfusing venous blood in the lungs of a cat, reduced the flow from 30 c.c. per min. to 24 c.c. per min.

A strong solution of chloroform (1 in 800) reduced the flow from 44 c.c. per min. to 20 c.c. per min. and after the chloroform was washed out by the Ringer solution the rate was again 44 c.c. per min. Then a stronger solution of chloroform (1 in 600) was tried. The rates of flow before, during and after the experiment were 44 c.c., 15 c.c., and 30 c.c. respectively. Therefore the first experiment did not produce rigor mortis.
(Figs. 12 - 20).

(C) With the vessels of the small intestine (cat, dog).

Leonard Hill[18] observed that chloroform more than any other known agent rapidly abolishes the neuro-vascular mechanism which compensates for the hydrostatic effect of gravity, by paralysing the splanchnic vasomotor tone and by paralysing the Respiratory /

Respiratory pump.

Embley and Martin[2] observed marked dilatation as the result of the action of chloroform upon the vessels of the bowel. They used defibrinated blood oxygenated by being previously passed through the lungs.

A loop of the small intestine was carefully separated and a cannula tied into the main artery of this loop. Into the cut ends of the loop were passed short glass tubes and a ligature tied round each tube so as to include the wall of the bowel. This prevented leakage from the cut vessels at the ends. In order to keep the apparatus free from intestinal contents the bowel was gently syringed out with warm Ringer's fluid previous to the experiment.

The experiments, performed under conditions similar to those employed by Embley and Martin i.e. with the use of highly oxygenated blood, confirm their results; thus the rate of flow through the vessels of a loop of small intestine was increased from 24 c.c. to 34 c.c. per min. by the action of chloroform (1 in 4000) acting for two minutes only. In my circulating apparatus only small quantities of chloroform solution could be added to the circulating fluid, thus limiting the action to a short time.

From /

From my experiments, with the apparatus described in Method No. 1, it was evident that the length of time of action of the chloroform does not materially alter the result.

When perfusing with Ringer's fluid or with venous defibrinated blood, constriction is the result of the action of chloroform (1 in 400 - 1 in 10,000) upon the blood vessels of the small intestine.

Fig.26 shows the effects of chloroform solutions of strengths 1 in 3000, 1 in 800 and 1 in 3000 respectively, Ringer's solution being the solvent fluid. Fig. 29 shows the constriction produced by a solution of chloroform in Ringer (1 in 6000) perfused for seven minutes under constant oxygen pressure as described in the first method of experimentation. (Figs. 21 - 31).

(D) With the vessels of the heart (dog).

Sherrington and Sowton[22] repeatedly noticed that chloroform solution slows the flow through the coronary circulation and attributed this slowing to the constriction of the coronary vessels.

Schäfer and Scharlieb[1] observed that the effect of chloroform upon the coronary arteries is to produce constriction.

The /

The heart of a dog was employed in my experiments, the cannula being tied into the left coronary artery and its area alone perfused. No experiment was attempted until the heart had ceased beating spontaneously.

The results resemble those obtained with the organs previously described. Fig. 33 shows the result when oxygenated defibrinated blood was the circulating fluid; and Fig. 37 when Ringer's fluid was used. Thus the coronary vessels behave like other vessels when under the action of chloroform, dilating when oxygenated blood is the medium and contracting when venous blood or Ringer's fluid is used.
(Figs. 32 - 38).

(E) With the vessels of the liver (cat).

The cannula was tied into the hepatic artery of the liver of a cat. When perfused with Ringer's fluid chloroform (1 in 200 - 1 in 10,000) constricts the hepatic vessels.

If defibrinated blood be used great difficulty is experienced in oxygenating the blood after it has once circulated through the liver vessels; that is to say that the defibrinated blood becomes venous much more rapidly than is the case when perfusing other organs /

organs. It is thus difficult to determine what action chloroform has on the hepatic vessels when oxygenated defibrinated blood is circulating through them. In only two experiments was the blood at all arterial in appearance and in these dilatation was observed.

It was however very evident that the constriction obtained varies with the degree of cyanosis of the defibrinated blood. At the beginning of the experiment chloroform (1 in 2500) seems to have little effect, but as the blood becomes more venous the same strength of chloroform produces a marked constriction.

Very strong solutions (1 in 800) acting for two minutes do not produce rigor mortis.
(Figs. 39 - 48).

(F) With the vessels of the Kidney (cat, dog.)

Schäfer and Scharlieb[1] observed that if strong solutions of chloroform are used the kidney vessels are constricted, whereas if weak solutions are used they are dilated.

Embley and Martin[2] found that the kidney vessels are dilated by anaesthetic quantities of chloroform.

Both kidneys of a cat were perfused by tying the cannula into abdominal aorta and ligaturing all the branches except the renals. In other experiments one kidney was taken from a dog.

I /

I find that strong solutions of chloroform (1 in 200 to 1 in 800) constrict the renal vessels. Weaker solutions up to 1 in 10,000 dilate these vessels. It does not make any difference to this result, which perfusion fluid is used, Ringer's fluid, oxygenated and venous defibrinated blood giving like effects.

Fig. 52 shows an experiment in which Ringer's solution was used and Fig. 50 one in which oxygenated blood was used; the chloroform solution was much weaker in the second experiment.

With Ringer's fluid, for the same strength of chloroform, the action is more marked than with defibrinated blood.

Fig. 56 shows the result of a solution of chloroform (1 in 1000) acting for fourteen minutes on the kidney vessels of a cat, the experiment being performed with the first apparatus described. The dilatation was very marked and rigor mortis was not produced. (Figs. 49 - 63.)

(G) With the vessels of the spleen (cat, dog.)

Gaskell and Shore[17] say that the volume of an organ such as the spleen does not increase when chloroform is given to the animal, as it would if a dilatation of its vessels or relaxtion of its musclar tissue occurs; but, on the contrary, it diminishes /

diminishes more than one can account for by the fall in general pressure, showing that there is some active constriction.

In my experiments with the spleen results, similar to those that are already described for the limbs, lungs, intestine and heart, were obtained.

With oxygenated defibrinated blood constriction was obtained with solutions of chloroform from 1 in 200 up to 1 in 2000, whereas in the above organs this was only obtained with solutions stronger than 1 in 1000. It has already been pointed out, that such strong solutions alter the blood and make it venous; probably this is why constriction is obtained. Weaker solutions (1 in 2000 to 1 in 10,000) produce dilatation.

With Ringer's fluid and venous defibrinated blood constriction is the result of the action of chloroform (1 in 400 to 1 in 8000).
(Figs. 64 - 71).

(H) With the vessels of the brain.

Roy and Sherrington[11] found that chloroform causes constriction rather than dilatation of the vessels of the brain when administered in the ordinary way by inhalation.

Hürthle[9] observed that the brain vessels dilate at /

at first as the direct effect of chloroform; this is followed shortly before death by constriction.

The brain of a sheep was dissected away from its attachments to the skull, the dura mater covering being kept intact. A cannula was tied into one (internal carotid) artery, all the others being ligatured.

Ten brains were perfused with Ringer's fluid under constant oxygen pressure. All strengths of chloroform solution (1 in 400 - 1 in 10,000) produce constriction of these vessels.

No experiments were done with defibrinated blood. (Figs. 72 - 74).

(I) With all the vessels (rat).

Schäfer and Scharlieb[1] perfused the frog's vessels with chloroform dissolved in Ringer's solution (1 in 200 to 1 in 20,000) and obtained constriction as the result.

I have performed a similar experiment with the vessels of a rat, the animal being killed by destroying the cervical region of the cord. The cannula was tied into the aorta and the pressure kept at 100 m.m. Hg. Marked constriction was observed with all strengths of chloroform solution (1 in 400 to 1 in 10,000). (Fig. 75.)

It is also evident, as the above observers pointed /

pointed out, that after weak solutions of chloroform had been in action for some minutes without effect, there is a tendency towards a gradual diminution in the rate of flow, which appears to be caused by oedema of the tissues. I found that, in the rat, this oedema eventually occurs even if pure Ringer is perfused; but if chloroform solution is used it occurs far more rapidly. The oedema is most marked in the splanchnic area, the abdomen becoming greatly distended.

During the gradual oedema the constricting effect of chloroform solutions in Ringer (1 in 400 to 1 in 10,000) can still be demonstrated.

CONCLUSIONS.

Chloroform, when perfused with defibrinated blood of arterial appearance, dilates the vessels of the limbs, lungs, small intestine, heart, liver, kidney and spleen.

When perfused with Ringer's fluid or venous defibrinated blood, it constricts the vessels of all the above organs with the exception of the kidney vessels; these are dilated.

When perfused with Ringer's fluid it also constricts the blood vessels of the brain.

REFERENCES TO LITERATURE

1. Schäfer and Scharlieb - Trans. Roy. Soc. Edin., Vol.XLI., 1904.
2. Embley and Martin - Journ. Physiol., Vol. XXXII., 1905.
3. Argyll Campbell - Quart. Journ. Exptl. Physiol., Vol. IV., 1911.
4. Harley - Proc. Physiol. Soc. London, 1865.
5. Scheinesson - Centralbl. fur med. Wiesenschaften, 1869.
6. Bowditch and Minot - Boston Med. and Surg. Journ. 1874.
7. Arloing - Thèse, Paris, 1879.
8. Newman - Journ. Anat. and Physiol., Vol. XIV., 1879.
9. Hürthle - Pflüger's Arch., Vol. XLIV., 1889.
10. Dastre - Les Anesthétiques. 1890.
11. Roy and Sherrington - Journ. Physiol., Vol. XI., 1890.
12. Wood - Brit. Med. Journ. Aug. 16th. 1890.
13. MacWilliam - Brit. Med. Journ. October, 1890.
14. Report of Hyderabad Chloroform Commission. 1891.
15. Hare and Thornton - Lancet. 1893.
16. Lauder Brunton - Brit. Med. Journ., Vol. I., 1893.
17. Gaskell and Shore - Brit. Med. Journ., Vol. I., 1893.
18. Leonard Hill - Brit. Med. Journ., April 1897.
19. Duplay and Hallion - Arch. Gen. de Med., Aug. 1900.
20. Committee Brit. Med. Assoc. - Lancet, 26th Jan., 1901.
21. Embley - Brit. Med. Journ. April 1902.
22. Sherrington & Sowton - Thompson Yates Lab. Reps., Vol. V., 1903.
23. Sherrington & Sowton - Brit. Med. Journ. 1906.
24. Bayliss - Proc. Roy. Soc., B. Vol. 80, 1908.
25. /

REFERENCES - continued :-

25. Leonard Hill - Text Book of Physiology, Vol. II., Schäfer, p. 132. 1900.

26. Lister - Quoted by Gaskell and Shore, Brit. Med. Journ., Vol. I., p. 225, 1893.

27. Meyer, O.B. - . . Zeitschr. f. Biol., XLVIII, 1906.

28. Langendorff, O. - Zentralbl. f. Physiol., XXI., 1907.

29. Dale, H.H., - Bio - chem. Journ., Vol. IV., 1909.

30. De Bonis & Susanna - Zentralbl. f. Physiol., XXIII., 1909.

31. Pal, J. - Wien. med. Wochenschr., 1909.

32. Brodie and Dixon - Journ. Physiol., Vol. XXX., 1904.

33. Wiggers - Pharmacol. and Exp. Therap. 1909.

34. Langendorff - Arch. fur Physiol. LXI., 1895.

A P P E N D I X

TABLES SHOWING DETAILS OF TYPICAL EXAMPLES OF ALL EXPERIMENTS.

(A) THE LIMBS.

Animal	Method	Perfusing Fluid	Pressure	Pulsations per min.	Temperature.	Strength of Drug.	Number of c.c. flowing from vein per minute, before, during and after action of drug. Before	During	After	Results.
Cat	No. 2	Oxygenated Blood 100%	120 mm.	94	37°C.	1 in 3,000	24	30	24	Dilatation.
"	"	" " 50%	140 mm.	94	38°C.	1 in 3,000	16	22	18	"
"	"	" " 100%	120 mm.	94	37°C.	1 in 3,000	22	26	22	"
"	"	" " 40%	130 mm.	94	36°C.	1 in 3,000	9	14	12	"
"	"	" " 30%	90 mm.	94	37°C.	1 in 2,500	32	36	32	"
						1 in 1,500	32	40	32	"
"	"	" " 50%	150 mm.	94	37°C.	1 in 1,500	14	16	14	"
"	"	Cyanotic Blood 50%	95 mm.	84	37.6°C.	1 in 3,000	24	20	24	Constriction.
						1 in 1,500	24	18	24	"
						1 in 800	24	10	24	"
"	"	" " 50%	90 mm.	84	39°C.	1 in 3,000	26	20	26	"
"	"	" " 50%	150 mm.	94	37°C.	1 in 400	16	10	16	"
"	"	Ringer's Fluid	80 mm.	94	36°C.	1 in 5,000	48	44	48	"
						1 in 1,400	48	40	48	"
						1 in 600	48	6	24	"
Rabbit	"	" "	110 mm.	84	41°C.	1 in 3,000	32	24	32	"
						1 in 1,600	32	20	32	"
Cat	"	" "	90 mm.	94	37°C.	1 in 2,500	34	32	34	"
						1 in 1,500	34	30	34	"
						1 in 700	28	20	28	"
						1 in 700	32	16	16	"
"	"	" "	120 mm.	84	38.5°C.	1 in 2,000	24	16	24	"
"	"	" "	120 mm.	84	39°C.	1 in 600	28	14	28	"
						1 in 600	30	20	30	"
"	No. 1	" "	100 mm.	-	36°C.	1 in 10,000	20	18	20	"
"	"	" "	"	-	37°C.	1 in 7,500	9	7	9	"
"	"	" "	"	-	40°C.	1 in 6,000	9	7	8	"
"	"	" "	"	-	37°C.	1 in 5,000	24	16	22	"
"	"	" "	"	-	36°C.	1 in 3,000	27	22	27	"
"	"	" "	"	-	35.5°C.	1 in 2,000	7	4	6	"
"	"	" "	"	-	39°C.	1 in 1,200	24	18	20	"
"	"	" "	"	-	36°C.	1 in 600	14	10	14	"

(B) THE LUNGS.

Animal	Method	Perfusing Fluid	Pressure	Pulsations per min.	Temperature	Strength of Drug.	Number of c.c. flowing from vein per minute, before, during and after action of drug. Before	During	After	Result.
Cat	No. 2	Oxygenated Blood 30%	30 mm.	94	37°C.	1 in 10,000	30	34	34	Dilatation.
"	"	" " 50%	40 mm.	94	36.5°C	1 in 10,000	20	26	20	"
						1 in 3,500	24	28	24	"
						1 in 2,500	28	32	24	"
						1 in 1,600	14	22	14	"
"	"	" " 50%	"	100	39°C.	1 in 8,000	64	72	66	"
"	"	" " 50%	30 mm.	94	37°C.	1 in 5,000	26	40	32	"
						1 in 2,500	24	32	26	"
"	"	" " 30%	30 mm.	94	38°C.	1 in 3,500	30	36	32	"
"	"	Cyanotic Blood 30%	"	"	"	1 in 3,500	30	24	30	Constriction
"	"	Ringer's Fluid	35 mm.	"	39°C.	1 in 7,000	52	48	52	"
						1 in 2,000	48	40	44	"
						1 in 800	44	20	44	"
						1 in 800	44	15	30	"
"	"	" "	30 mm.	"	39°C.	1 in 2,500	20	16	24	"
"	"	" "	"	"	37°C.	1 in 600	28	12	28	"
"	No. 1	" "	"	"	39°C.	1 in 10,000	24	18	24	"
"	"	" "	"	-	36°C.	1 in 5,000	24	16	26	"
"	"	" "	"	-	37°C.	1 in 3,000	48	42	48	"
"	"	" "	"	-	39°C.	1 in 2,000	30	18	28	"
"	"	" "	"	-	35.5°C	1 in 600	27	15	27	"

(C) THE SMALL INTESTINE.

Animal	Method	Perfusing Fluid	Pressure	Pulsations per min.	Temperature.	Strength of Drug.	Number of c.c. flowing from vein per minute, before, during and after action of drug. Before	During	After.	Result.
Cat	No. 2.	Oxygenated Blood 33%	120 mm.	94	39°C.	1 in 10,000	32	36	32	Dilatation
"	"	" " 30%	90 mm.	"	38°C.	1 in 8, 000	40	46	42	"
"	"	" " 33%	140 mm.	"	39°C.	1 in 6,000	36	40	36	"
						1 in 4, 000	24	34	32	"
"	"	" " 40%	90 mm.	96	37°C.	1 in 3,000	16	26	26	"
"	"	Cyanotic Blood 50%	100 mm.	94	37°C.	1 in 3,000	24	20	24	Constriction
						1 in 1,200	22	12	22	"
"	"	Ringer's Fluid	82 mm.	"	38°C.	1 in 8,000	40	36	40	"
"	"	" "	80 mm.	"	37°C.	1 in 800	40	12	40	"
"	No. 1.	" "	100 mm.	-	35°C.	1 in 10,000	14	12	14	"
"	"	" "	"	-	"	1 in 8,000	12	9	12	"
"	"	" "	"	-	"	1 in 6,000	15	10	18	"
"	"	" "	"	-	34.5°C	1 in 4,000	14	10	14	"
"	"	" "	"	-	37°C.	1 in 3,000	9	8	10	"
"	"	" "	"	-	35°C.	1 in 2,000	10	8	9	"
"	"	" "	"	-	36°C.	1 in 1,200	60	36	60	"
"	"	" "	"	-	36.5°C.	1 in 1,000	48	36	48	"
"	"	" "	"	-	35°C.	1 in 800	36	24	32	"

(D) THE HEART

Animal	Method	Perfusing Fluid	Pressure	Pulsations per min.	Temperature.	Strength of drug	Number of c.c. flowing from vein per minute, before, during and after action of drug. Before	During	after.	Result.
Dog	No. 2	Oxygenated Blood 50%	130 mm.	90	38°C.	1 in 7,000	26	32	30	Dilatation.
"	"	" " 30%	100 mm.	106	40°C.	1 in 6,000	36	42	42	"
"	"	" " 50%	90 mm.	94	36.5°C.	1 in 4,000	24	30	24	"
"	"	" " 50%	120 mm.	94	37°C.	1 in 2,500	22	26	24	"
"	"	Cyanotic Blood 30%	100 mm.	106	38°C.	1 in 6,000	38	32	38	Constriction
"	"	" " 40%	100 mm.	94	36°C.	1 in 4,000	48	42	48	"
"	"	" " 40%	100 mm.	94	36°C.	1 in 1,000	46	36	48	"
"	"	Ringer's Fluid	"	106	40°C.	1 in 6,000	48	40	48	"
"	"	" "	"	94	39°C.	1 in 4,000	28	24	28	"
"	No. 1	" "	"	-	37°C.	1 in 10,000	12	10	12	"
"	"	" "	"	-	36°C.	1 in 4,000	42	30	36	"

(E) THE LIVER.

Animal	Method	Perfusing Fluid	Pressure	Pulsations per min.	Temperature	Strength of drug.	Number of c.c. flowing from vein per minute, before, during and after action of drug.			Results
							Before	During	After	
Cat	No.2	Oxygenated Blood 30%	120 mm.	94	39°C.	1 in 4,000	14	20	24	Dilatation.
"	"	" " 30%	140 mm.	94	38°C.	1 in 3,000	27	30	28	"
"	"	Cyanotic Blood 50%	110 mm.	94	39°C.	1 in 5,000	16	12	16	Constriction
"	"	" " 30%	120 mm.	94	39°C.	1 in 4,000	22	16	22	"
"	"	Ringer's Fluid	110 mm.	94	39°C.	1 in 4,000	16	12	16	"
"	"	" "	150 mm.	94	38°C.	1 in 800	24	12	24	"
"	No.1	" "	100 mm.	-	35°C.	1 in 10,000	30	27	28	"
"	"	" "	"	-	37°C.	1 in 5,000	14	12	14	"
"	"	" "	"	-	35°C.	1 in 3,000	18	14	18	"
"	"	" "	"	-	37°C.	1 in 1,000	14	12	14	"
"	"	" "	"	-	37°C.	1 in 400	16	4	14	"

(F) THE KIDNEYS.

Animal	Method	Perfusing Fluid	Pressure	Pulsations per min.	Temperature.	Strength of Drug.	Number of c.c. flowing from vein per minute, before, during and after action of drug. Before	During	After.	Result
Dog	No. 2	Oxygenated Blood 30%	100 mm.	106	39°C.	1 in 6,000	20	24	20	Dilatation
"	"	" 100%	120 mm.	106	37°C.	1 in 5,000	20	24	22	"
"	"	" 30%	120 mm.	74	37°C.	1 in 3,000	20	24	20	"
"	"	Ringer's Fluid	100 mm.	106	39°C.	1 in 3,000	32	40	34	"
						1 in 1,000	34	44	34	"
"	"	" "	130 mm.	84	38°C.	1 in 800	18	14	18	Constriction
Cat	No. 1	" "	100 mm.	-	36°C.	1 in 10,000	6	8	7	Dilatation.
"	"	" "	"	-	36°C.	1 in 7,500	6	9	6	"
"	"	" "	"	-	35°C.	1 in 6,000	18	24	22	"
"	"	" "	"	-	39°C.	1 in 5,000	8	15	12	"
"	"	" "	"	-	38.5°C	1 in 3,000	7	9	7	"
"	"	" "	"	-	39°C.	1 in 2,000	21	25	21	"
"	"	" "	"	-	36.5°C	1 in 1,200	8	14	10	"
"	"	" "	"	-	38.5°C	1 in 1,000	4	10	6	"
"	"	" "	"	-	38.5°C	1 in 800	8	10	8	"
"	"	" "	"	-	39°C.	1 in 600	9	7	9	Constriction.
"	"	" "	"	-	38°C.	1 in 400	16	8	16	"
"	"	" "	"	-	37°C.	1 in 200	24	16	24	"

(G) THE SPLEEN

Animal	Method	Perfusing Fluid	Pressure	Pulsations per min.	Temperature	Strength of Drug.	Number of c.c. flowing from vein per minute, before, during and after action of drug. Before	During	After	Result.
Dog	No. 2	Oxygenated Blood 30%	120 mm.	106	40°C.	1 in 6,000	16	24	22	Dilatation.
"	"	" " 40%	150 mm.	106	39°C.	1 in 3,000	24	18	24	Constriction
"	"	Ringer's Fluid	120 mm.	106	40°C.	1 in 3,000	28	22	28	"
						1 in 600	28	14	28	"
Cat	No. 1	" "	100 mm.	-	37°C.	1 in 8,000	10	8	9	"
"	"	" "	"	-	37°C.	1 in 6,000	8	6	8	"
"	"	" "	"	-	37°C.	1 in 5,000	15	12	14	"
"	"	" "	"	-	37°C.	1 in 600	14	9	14	"
"	"	" "	"	-	39°C.	1 in 400	12	4	12	"

(H) THE BRAIN

Sheep	No. 1	Ringer's Fluid	100 mm.	-	36°C.	1 in 10,000	9	7	9	Constriction
"	"	" "	"	-	37°C.	1 in 6,000	12	10	12	"
"	"	" "	"	-	36°C.	1 in 5,000	14	12	14	"
						1 in 2,500	12	10	12	"
						1 in 1,200	10	8	9	"
						1 in 400	6	4	4	"
"	"	" "	"	-	35°C.	1 in 3,000	16	14	16	"
"	"	" "	"	-	37.5°C.	1 in 600	16	6	10	"

(I) RAT (entire vascular system)

Rat	No. 1	Ringer's Fluid	100 mm.	-	37°C.	1 in 10,000	12	10	12	Constriction
						1 in 5,000	12	9	12	"
						1 in 1,000	12	8	9	"
						1 in 400	9	6	6	"
"	"	" "	"	-	37°C.	1 in 8,000	18	15	16	"